2919-Pos Board B74
The Sequence and Function Relationship in Ligand-Induced Folding
Christopher Eginton, Dorothy Beckett.
University of Maryland, College Park, MD, USA.
The Escherichia coli biotin repressor, BirA, the central protein of the E. coli Biotin Regulatory System serves as a metabolic enzyme and the transcription repressor of the biotin biosynthetic operon. For both functions, the active BirA species is bound to bio-5'-AMP, which it synthesizes from substrates biotin and ATP. As an enzyme, BirA catalyzes post-translational biotin linkage to a specific lysine residue of the biotin carboxyl carrier protein (BCCP), a subunit of acetyl-CoA carboxylase, which catalyzes the first committed step in fatty acid biosynthesis. Transcription repression requires self-association of the adenylate-bound BirA followed by site-specific DNA binding to the biotin operator of the biotin biosynthetic operon. Both functions require folding of two flexible surface loops including the biotin-binding loop (BBL), comprised of residues 116-124, that folds over the biotin moiety and the adenylate-binding loop (ABL), comprised of residues 212-234, that folds over the adenine moiety of the adenylate. In the BirA: adenylate structure side chains of residues F124 and P126 of the BBL and M211 and V218 of the ABL bridge the two loops. Combined kinetic and equilibrium measurements of the consequences of single alanine substitutions of these bridging residues indicate heterogeneity in the effects on the multiple BirA functions. Isothermal titration calorimetry and stopped-flow fluorescence measurements reveal that the substitutions significantly compromise both bio-5'-AMP binding and synthesis. By contrast, these same substitutions have modest effects on homodimerization and biotin transfer to BCCP. These results in combination with previous studies of amino acid replacements in three ABL residues that form a hydrophobic cluster concomitant with adenylate binding indicate a ligand-linked folding process that is characterized by a complex relationship between sequence and function.

2920-Pos Board B75
Structural Architecture of the Multi-Immunoglobulin-Like Domain Chain from Ligb
Ching Lin Hsieh[1], Christopher P. Ptak[1], Alexander S. Maltsev[2], Robert E. Oswald[1], Yung Fu Chang[1].
[1]Cornell University, Ithaca, NY, USA, [2]National Institutes of Health, Bethesda, MD, USA.
Surface proteins on pathogenic strains of Leptospira are involved in host adhesion interactions, an important step in bacterial infection. Lig (Leptospira immunoglobulin (Ig) -like) proteins can bind to human extracellular matrix proteins and have shown promise in typing leptospiral isolates for pathogenesis. Lig proteins are composed of twelve to thirteen Ig-like domain repeats that are anchored to the outer membrane at the N-terminus. Here, we combine experimental data from small-angle x-ray scattering (SAXS) and nuclear magnetic resonance (NMR) to develop a model of the overall architecture of the multi-Ig-like domain chain from LigB. SAXS scattering curves were obtained from constructs containing two to five tandem domains. An ensemble of low resolution envelopes was reconstructed and combined to create a low resolution model of the twelve domains from LigB. In addition, we solved the high resolution NMR structure of the twelfth Ig-like domain from LigB (LigB12). using LigB12-dervied homology models for the other eleven domains, LigB1-11, along with additional experimentally derived NMR constraints, we generated a high resolution model for the Ig-like domain region of LigB. The LigB Ig-like domain architecture should prove useful in understanding the Leptospira surface structure, Lig protein mediated host interactions, and Lig protein accessibility to antibodies.

2921-Pos Board B76
Hexokinase Monomer-Dimer Preferential Hydration Determined by SANS and Osmotic Stress
Christopher B. Stanley[1], Hugh O'Neill[1], Erica Rowe[1,2], Valerie Berthelier[2].
[1]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]University of Tennessee Medical Center, Knoxville, TN, USA.
Yeast hexokinase (HK) exists in equilibrium between the monomer and homodimer states, which can be modulated by solution pH, ionic strength, temperature, protein concentration, and ligand binding. HK monomer-dimer equilibrium plays a regulatory role but its importance to function is not entirely clear. To directly correlate protein structure with the associated hydration and energetics, we are using small-angle neutron scattering (SANS) coupled with osmotic stress. SANS allows three regions of contrast to be monitored upon osmolyte addition: protein, protein-associated water that excludes osmolyte, and bulk water/osmolyte solution. We find the preferential hydration of HK monomer and dimer states to depend on the osmolyte chemistry and size. However, the difference in hydration between the monomer and dimer remains independent of the particular osmolyte. This research should prove useful in characterizing the forces governing the burial of complementary protein interfaces, which are especially important for weak types of protein recognition and association mechanisms that are often difficult to study experimentally.

2922-Pos Board B77
Intravital and In Vitro Time-Lapse Imaging Reveals Microtubule Dynamics in Skeletal Muscle Fibers
Sarah Oddoux.
NIH, Bethesda, MD, USA.
Microtubules (MTs) play an essential role in dynamic cell processes but they also play a mechanical role, buttressing cells against compressive forces. Both roles may be especially important in the very large skeletal muscle fibers; it has recently been shown that microtubules are part of the pathology of Duchenne Muscular Dystrophy. However, the organization of microtubules in muscle fibers is poorly understood. They do not resemble the microtubule asters of proliferating cells; instead they form complex three-dimensional networks without clear organizing centers (MTOC). Imunofluorescence with anti-tubulin or anti-EB3 (microtubule plus-end marker) does not suggest a dynamic behavior.
To understand how muscle microtubules are organized, we have expressed GFP-tubulin and EB3-GFP in the mouse footpad Flexor Digitorum Brevis (FDB) muscle. Time-lapse sequences were recorded from single FDB fibers plated on dishes, and from the intact muscle intravitally. Both approaches showed dynamic EB3 spots (8 and 4 micrometer/min respectively, not significantly different) and a predominantly stable tubulin network. Simultaneous observation of mCherry-tubulin and EB3-GFP indicated that EB3 moves along tracks of stable microtubules. Super-resolution microscopy confirmed that muscle microtubules include longitudinal and transverse bundles. Observation of recovering microtubules after nocodazole treatment shows that they originate from nuclear membranes and from cytoplasmic Golgi elements (50%) both of which show the MTOC protein pericentrin. The tools developed have allowed us to see for the first time how microtubules form and move in live muscle fibers and provide a platform for exploring the role of microtubules in muscle and the consequences of their defects in muscle diseases.

2923-Pos Board B78
Characterization of the Interaction between Rheb and a Truncated Construct of Tuberous Sclerosis Complex 2 (TSC2) and the Potential Effect of Heparin on TSC2-Stimulated GTP Hydrolysis
Kyla Morris, Paul D. Adams.
University of Arkansas, Fayetteville, AR, USA.
Rheb is a member of the Ras superfamily of proteins, a known regulator of cell growth, proliferation, and signaling. Interactions involving Rheb and the TSC2 protein regulate the cycling of Rheb between its nucleotide-bound states and, thereby, cell growth. The C-terminus of TSC2 contains a ''perceived'' conserved GAP (GTPase-activating protein)-binding domain. Rheb binds to TSC2 and this binding facilitates TSC2-stimulated Rheb GTP hydrolysis to regulate cell signaling. At present, little is known about the molecular features of the Rheb-TSC2 protein interaction. We present biochemical and biophysical data on the interaction of Rheb with TSC2, using a shorter, 218 amino acid GTPase derivative of TSC2 (TSC2-218) that has been used to characterize the GTP hydrolysis rate of activated Rheb.
Heparan sulfate is a derivative of heparin, a sulfonated polysaccharide known to interact with diverse proteins and, thus, biologically important. TSC2-218 contains a polybasic region and preliminary studies indicate that heparan sulfate may be capable of binding to TSC2-218, but not Rheb. We present evidence that the binding of heparan sulfate to TSC2-218 does not interfere with TSC2-218-Rheb binding and may enhance the ability of TSC2-218 to stimulate Rheb GTP hydrolysis. NMR spectroscopy was used to outline TSC2 residues that might be important for interactions with Rheb and define conformational changes induced in TSC2-218 by the presence of heparan sulfate as defined by chemical shift changes. This work highlights molecular features of the Rheb-TSC2 interaction that may contribute to our understanding of new ways to regulate abnormal cell signaling activity facilitated by the reduced GTP-GDP cycling of Rheb in the presence of disease-causing mutants of TSC2.

2924-Pos Board B79
Designing Self Assembling Beta Helices
Ellen M. White, Krishna Kishore Inampudi, Corey J. Wilson, Andrew D. Miranker.
Yale University, New Haven, CT, USA.
Amyloid fibers are unusually stable structures that self-assemble from soluble proteins. The fibers are typically composed of parallel β-sheets that run along